
Effect of Temulawak (*Curcuma xanthorrhiza* Roxb.) Extract on the MDA Levels and GPx Activity in the Brains of Trimethyltin Induced Dementia Model Rats

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Effect of Temulawak (*Curcuma xanthorrhiza* Roxb.) Extract on the MDA Levels and GPx Activity in the Brains of Trimethyltin Induced Dementia Model Rats

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Oxidative stress in the brain plays an important role in the pathogenesis of dementia. The rhizome of temulawak (*Curcuma xanthorrhiza* Roxb.) contains curcumin and xanthorrhizol which possess antioxidant activity and thus has the potential to deter oxidative stress. The study aims to identify the effect of the ethanol extract of a temulawak rhizome on the malondialdehyde (MDA) levels and glutathione peroxides (GPx) activity in the brains of trimethyltin (TMT) induced dementia model rats. The study employed 30 adult male Sprague Dawley rats divided into six groups, each consisting of five rats. The normal group was given a CMC-Na 1% solution orally, the TMT group also received a CMC-Na 1% solution orally, while the treatment groups were orally given temulawak rhizome extract (TRE) with doses of 50 (E50), 100 (E100) and 150 mg/kg bw (E150), and 200 mg/kg bw of citicoline (Cit200), respectively. Before treatment, all groups, except the normal group, were injected with single doses of 8 mg/kg bw of TMT intraperitoneally. The treatment was administered for 21 days. On the 29th day the rats were sacrificed, their brains were retrieved and had their hemispheric cerebrum separated for the measurement of the MDA levels and GPx activity in its homogenate. Data of the MDA levels and GPx activity were statistically analyzed by a one-way ANOVA test followed by a posthoc LSD test. Results showed that TMT injection could raise the MDA levels and lower GPx activity ($p<0.05$). The administration of TRE with a dose of 50 mg/kg bw was able to inhibit increase in MDA levels and augment GPx activity in the brain but had significant difference from the normal group ($p<0.05$). Meanwhile, the administration of 100 and 150 mg/kg bw doses of TRE could prevent rise in MDA levels and enhance GPx activity of the brain ($p<0.05$) with no meaningful difference from the normal group and the group given citicoline ($p>0.05$). It can be concluded from these results that TRE in doses of 100 and 150 mg/kg bw can avert oxidative stress in TMT induced dementia model rats.

Keywords: *Curcuma xanthorrhiza* Roxb., trimethyltin, MDA, GPx, dementia

1. INTRODUCTION

Dementia is a term used to describe a collection of memory disorder symptoms such as forgetfulness, disorientation, communication difficulties, and the declining capability to analyze and make decisions¹. Oxidative stress is the imbalance between free radicals and antioxidants, a significant pathogenesis in the development of dementia². Increasing oxidative stress prompts brain neuron degeneration which may cause dementia^{3,4}. Brain cells are highly vulnerable to oxidative damage as the brain is an organ which greatly requires oxygen. The levels of unsaturated fatty acids in the brain are high, but its antioxidant defense system is relatively weaker than in other organs⁵. Oxidative damage to lipids and proteins may lead to functional and structural disorders of cell membranes, enzyme inactivity and eventually cell death⁶. Oxidative damage to lipids produces malondialdehyde (MDA), 4-hydroxy-2, 3-nonenal (HNE), acrolein, etc. Endogenous antioxidants in the body such as glutathione peroxidase (GPx) contribute in preventing such oxidative damage⁷.

Temulawak (*Curcuma xanthorrhiza* Roxb.) is a medicinal herb widely used as raw ingredient in the *jamu* (traditional medicine) and pharmaceutical industries. Several conducted studies have shown that temulawak contains curcumin and xanthorrhizol compounds which are recognized as potential antioxidants^{8,9}. Antioxidant activity has proved its function in abating oxidative damage and memory deficit related to neuron degenerative disorders including dementia¹⁰. The purpose of this research is to identify the effect of temulawak rhizome extract on the levels of malondialdehyde (MDA) and the activity of glutathione peroxidase (GPx) in the brains of trimethyltin (TMT) induced dementia model rats. It is expected that this study will serve as basis for the development of natural ingredients, particularly temulawak, as a dementia deterrent.

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2. EXPERIMENTAL DETAILS

Animals

The animals employed were Sprague Dawley male rats, weighing 150-250 g and aged 2.5 to 3 months, obtained from the Integrated Research and Testing Laboratory (LPPT) of Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. The test animals were treated in a laboratory condition of 25 to 26°C temperatures, 60-65% humidity with a light exposure cycle of 12 hours of bright and dark, and received feed and water ad libitum.

Materials

Dried temulawak rhizome simplicia was procured from CV. Merapi Farma, Yogyakarta. Chemicals comprising silica gel 60 F254, chloroform, methanol, ethanol 96%, NaCl 0.9%, CMC-Na 1%, and TMT were bought from Sigma-Aldrich. Citicoline was purchased from PT. Bernofarm, while other chemicals namely KCl, sodium dodecyl sulfate, acetic acid solution, n-butane, pyridine, NaOH, thiobarbituric acid, and 1,1,3,3-tetraethoxypropane (TEP) were bought from the Food and Nutrition Laboratory, Universitas Gadjah Mada, Indonesia.

Experimental Procedures

Extraction and standardization of temulawak extract

Temulawak rhizome extract was acquired by applying a maceration method with ethanol 96%. Identification of the curcumin compound in the extract was done by thin layer chromatography (TLC) using the mobile phase of chloroform and methanol (with a ratio of 9:1) and the stationary phase of silica gel 60 F254. Afterwards, the curcumin levels in the extract were determined using a densitometer with a wavelength of 426 nm. In addition, examination was also performed on the non-specific parameters of the extract, including the water levels, the total ash levels and the acid-insoluble ash levels.

Experimental design

All stages in this study have been approved by the Ethics Commission for Preclinical Research at LPPT UGM with article number 147/KEC-LPPT/V/2014.

As many as 30 rats that had been adapted for a week were split into six treatment groups, each consisting of five rats. The normal group orally received a CMC-Na 1% solution, the TMT group was also given a CMC-Na 1% solution orally, and the extract group was given the temulawak rhizome extract, each with a dose of 50 mg/kg bw (E50), 100 mg/kg bw (E100), and 150 mg/kg bw (E150), respectively. The next group received 200 mg/kg bw of citicoline orally. Prior to treatment, all groups were intraperitoneally injected with 8 mg/kg bw of TMT with the exception of the normal group. Treatment was administered for 21 days and on the 29th day the rats were sacrificed by means of CO₂ gas. The brain tissue was then retrieved and the hemispheres separated for the measurement of the MDA levels and GPx activity in the brain.

Brain MDA level measurement

The brain MDA level measurement was conducted per procedure utilized by Colado et al.¹¹. The MDA levels were gauged based on TBA reaction using a spectrophotometer at 532 nm wavelength with tetra ethoxy propane (TEP) as standard solution. The MDA levels were presented in nmol/mg tissue units.

Brain GPx enzyme activity measurement

Brain GPx activity was measured following a method by Wendel¹². GPx reduced cumene hydro peroxide which

oxidized GSH into GSSG. The GSSG was then reduced into GSH by glutathionereductase with NADPH. Decline in NADPH levels directly proportional to GPx activity was determined by spectrophotometer at 340 nm wavelength. GPx activity was presented in mU/mg protein units.

Protein determination

Protein levels were gauged with Lowry's method¹³ using bovine serum albumin (BSA) (1 mg/mL) as standard.

Data analysis

Data of MDA levels and GPx activity were statistically analyzed with a one-way ANOVA test followed by post hoc LSD test. Significance of results was established if $p < 0.05$.

3. RESULTS AND DISCUSSION

Temulawak Rhizome Extract

The curcumin content of the temulawak rhizome extract was identified by thin layer chromatography. The chromatogram profile is presented in Figure 1.

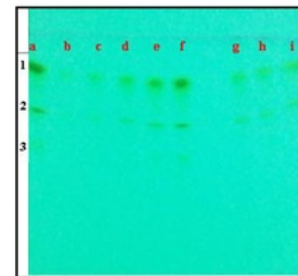


Figure 1. TLC profile of standard curcumin and temulawak rhizome extract. Legend: spot 1 (Rf 0.78) = curcumin; spot 2 (Rf 0.58) = desmethoxy curcumin; spot 3 (Rf 0.49) = bisdemethoxy curcumin; spot a-f = standard curcumin; and spot g-i = temulawak rhizome extract

Figure 1 indicates that temulawak rhizome extract contains curcumin. The subsequent results of temulawak rhizome extract standardization conducted in accordance with the Indonesian Herbal Pharmacopeia can be seen in Table I.

Table I. Temulawak rhizome extracts standardization results

Testing	Level terms as per the Indonesian Herbal Pharmacopeia	Obtained levels (%)
Curcumin levels	$\geq 14.20\%$	17.59 ± 0.48
Water levels	$< 10\%$	8.36 ± 0.27
Total ash levels	$\leq 7.8\%$	5.15 ± 0.30
Acid-insoluble ash levels	$\leq 1.6\%$	0.46 ± 0.03

The standardization results in Table I show that the temulawak ethanol extract used in this study fulfils the quality standards of the Indonesian Herbal Pharmacopeia.

Brain MDA level and GPx activity

The MDA level and GPx activity of the rats' brains are displayed in Figure 2 and 3.

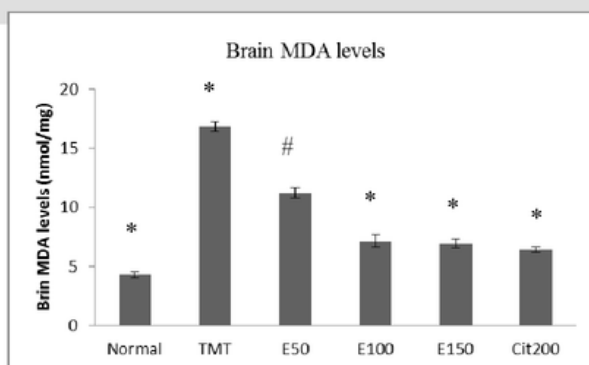


Figure 2. Levels of malondialdehyde (MDA) in all treatment groups of TMT induced dementia model rats. Legend: * $p < 0.05$ significantly different from TMT group, # $p < 0.05$ significantly different from normal group.

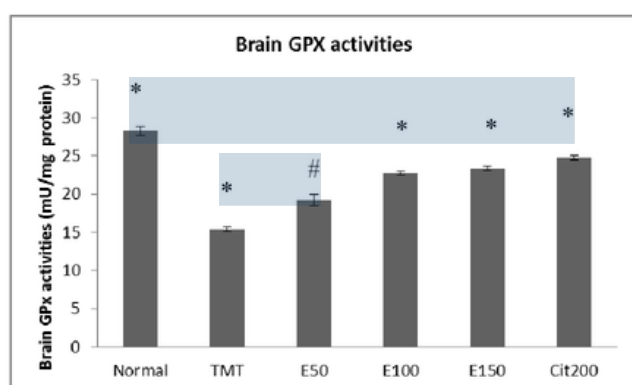


Figure 3. Brain glutathione peroxidase (GPx) activities in all treatment groups of TMT induced dementia model rats. Legend: * $p < 0.05$ significantly different from TMT group, # $p < 0.05$ significantly different from normal group.

Statistical analysis by one-way ANOVA and posthoc LSD test revealed that TMT 8 mg/kg bw injections could raise MDA levels and lower GPx activities in the rats' brains significantly when compared with the normal group ($p < 0.05$). MDA is a compound resulting from a non-enzymatic process and is the end product of lipid peroxidation. MDA measurement can become an indicator of oxidative damage affected by the presence of lipid peroxides due to an increase of free radicals¹⁴. Exposure to TMT has been known to intensify the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) of free radicals¹⁵ and lessen the activity of endogenous antioxidants, including GPx, thus prompting oxidative stress conditions. Augmentation of ROS and RNS will enhance glutathione oxidation, lipid peroxidation and oxidation of protein^{16,15}. TMT has also been proven to bring about selective degeneration in the central nervous system leading to selective neuronal death, particularly at the hippocampus^{17,18}. The hippocampus is the part of the brain involved in memory processing. TMT intoxication in test animals has been largely used to generate models of neurodegenerative disorders such as Alzheimer dementia¹⁹⁻²¹.

The administration of temulawak rhizome extract in doses of 50, 100, and 150 mg/kg bw was able to deter rises of MDA levels and GPx activity in the brain. More specifically, the administration of temulawak rhizome extract in doses of 100 and 150 mg/kg bw could preclude increases in cerebral MDA and GPx activity with no meaningful difference from the normal group ($p > 0.05$). Certain studies have pointed out that the main active components of

temulawak are curcuminoids and xanthorrhizol. The primary component of curcuminoids, curcumin has protective effects on lipid peroxides and hence decreases MDA levels^{22,23}. Curcumin possesses the activity of a strong antioxidant which can inhibit the growth and spread of free radicals, consequently curbing the progression of neuron injury in Alzheimer dementia^{24,25}. Curcumin can also increase glutathione peroxidase (GPx) enzymes because it is composed of phenolic hydroxyl groups, which are able to bind free radicals especially hydroxyl ones²⁶. With the development of GPx enzymes, hydro peroxides are reduced into water and glutathione is reduced as well, to the extent that the growth of highly reactive hydroxyl radicals can be avoided²⁷. Preclinic test results also show that xanthorrhizol contained in temulawak exhibits potential neuro protective effects and strong antioxidant activity by restraining lipid peroxidation in the homogenate of a murine brain injected with glutamate⁸.

In this study the administration of 200 mg/kg bw of citicoline divulged the ability to suppress increase in MDA levels and enhance brain glutathione peroxidase activity with no meaningful difference from the normal group. Citicoline has been proven as a neuroprotector that can prevent lipid peroxidation in nerve cells and stimulate glutathione synthesis²⁸. The development of glutathione as the enzyme substrate of glutathione peroxidase improves the activity of glutathione peroxidase enzymes, which act as antioxidants in the body, and decrease MDA levels.

4. CONCLUSION

It can be concluded from the research findings that temulawak rhizome extract at doses of 100 and 150 mg/kg bw can stifle escalations in MDA levels and GPx activities in the brain of a TMT induced dementia model rat. Other biochemical parameter measurements are required in future studies in order to identify the mechanism of temulawak extract in preventing dementia.

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